WEST NILE VIRUS
PROGRAM COMPLIANCE GUIDE
for
U.S. NAVY and MARINE CORPS
INSTALLATIONS
2003

Navy Disease Vector Ecology and Control Centers

Jacksonville, Florida

&

Bangor, Washington

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1. About West Nile Virus

Since West Nile virus (WNV) was first isolated in 1937, it has been known to cause human illness in Africa, West Asia, and the Middle East. Human and animal infections were first documented in the Western Hemisphere in 1999 in the New York City metropolitan area, New Jersey, and Connecticut. In the years 1999-2000, 83 human cases of West Nile illness were reported, and nine people died. In 2001, 66 human cases and nine deaths were reported from 10 States. In 2002, WNV had spread to most eastern and mid-western states, resulting in over 3,700 reported human cases and 254 deaths. Most deaths occurred in people with suppressed immune systems, especially the elderly. For example, in 2002 the median age of people diagnosed with WNV was 56, while the median age for fatal cases was 78. To date (2003), WNV infection has not been documented in any active duty member or dependent

Most people exposed to WNV, especially children and healthy, young adults, do not develop symptoms. A small proportion of people infected develop mild symptoms that include flu-like illness characterized by fever, muscle aches, swollen glands and skin rash. Less than one percent of infected people develop more severe illness that includes meningitis (inflammation of the spinal cord) or encephalitis (inflammation of the brain). Symptoms can also include headache, high fever, neck stiffness, stupor, disorientation, coma, tremors, convulsions, muscle weakness, and paralysis. Of the few people who develop meningitis or encephalitis, a small proportion die. Overall, death is estimated to occur in less than one out of 1,000 infections. Consistent with European outbreaks, human mortality due to WNV infection is rare in people under 50 years old.

West Nile Virus is transmitted to humans almost exclusively through the bite of an infected mosquito. The most common mosquitoes associated with WNV transmission are *Culex* species, although other species have been implicated in transmission. Mosquitoes become infected only when they feed on birds infected with WNV. Infected mosquitoes can then transmit WNV when they feed on humans or other animals. Birds serve as the reservoir for WNV. Other animals may become ill with the virus, but mosquitoes are not able to pick up the virus from any animal except birds. Although WNV has been found mainly in American crows and blue jays, it has been detected in more than 80 other species of birds, 22 mosquito species, and a variety of mammals. Among domestic animals, horses are especially susceptible, and illness in horses often serves as an indication of virus activity in an area. The virus cannot be transmitted from person to person by mosquitoes - only from a bird to a person. There is some evidence that handling live or dead infected birds can cause human infection, but for all practical purposes, it must be transmitted to humans by a mosquito. By controlling mosquitoes, WNV transmission to humans (and to birds and other animals) can be reduced or eliminated.

West Nile virus surveillance on Navy and Marine Corps (N/MC) installations began in May of 2002. Mosquitoes and birds having WNV infections have been found on N/MC installations, and WNV-positive horses have been found as well. On installations where positive animal samples are found, increased surveillance and mosquito control efforts reduce the risk of human infection.

2. Authority and Purpose of this Guide

Due to the rapid spread of WNV throughout nearly the entire U.S. in 2002, Navy policy concerning WNV now states that “all installations in States where WNV has been found shall conduct surveillance for mosquitoes, and control measures as needed, in conjunction with local/State public health departments” (GENADMIN/ SECNAV/271542ZSEP2002). Since WNV activity has now been reported from every State in the continental United States except Oregon, Nevada, Utah and Arizona, most CONUS naval installations are affected by this policy.

The medical department on each installation is ultimately responsible to that installation’s Commanding Officer for the health of personnel on that installation and for informing the installation Commanding Officer of health risks on that installation. This includes the local risk of vector-borne disease.

BUMED interprets this guidance to mean that installations should have a written plan to (a) assess WNV risk on their installation, (b) conduct WNV surveillance throughout the mosquito breeding season in their region, (c) take appropriate preventive and responsive action to reduce WNV risk to their active duty and dependent populations, and (d) report their activities to the proper authorities.

The present guide is intended to assist installations in preparing and executing the requirements listed in the preceding paragraph. This guide was prepared by the Navy Disease Vector Ecology and Control Centers in Jacksonville, Florida (DVECC Jax) and Bangor, Washington (DVECC Bangor), whose mission is to provide guidance for disease vector surveillance and control programs for the Navy and Marine Corps, worldwide. This document is intended for all Navy and Marine Corps installations in the continental United States. Because each military installation is unique, this guide is meant only to provide general guidance. We strongly recommend that any individual installation's WNV surveillance program be planned and executed in close consultation with DVECC Jax (for installations east of the Mississippi River) or DVECC Bangor (for western installations.)

Points of contact for the DVECCs and other agencies mentioned in this guide can be found in Appendix A

3. WNV Surveillance and Protection Plan

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The Public Works Department (PWD) of every military installation is required to maintain an Installation Pest Management Plan (IPMP.) Navy and Marine Corps installations are additionally required to maintain an Emergency Vector Control Plan (EVCP), which reinforces the role of BUMED in vector-borne disease suppression and is typically included as an appendix to the IPMP. If your installation is without either of these documents, contact your cognizant DVECC for assistance.

Your installation's WNV Surveillance and Protection Plan (WNVSPP) should follow the installation's EVCP guidelines for any potential mosquito-borne disease outbreak. The surveillance must detail the species of mosquitoes inhabiting the particular installation, baseline population and density levels, breeding areas, habitation zones, and effectiveness of vector control measures. Preventive medicine personnel should draw up the WNVSPP in consultation with their cognizant DVECC. The installation EVCP may already contain information regarding mosquito-borne disease on the installation. The EVCP should be modified to include the installation WNVSPP.

The WNVSPP should include dead bird surveillance, mosquito breeding site surveillance, breeding site elimination or treatment, larval mosquito surveillance and control, adult mosquito surveillance and control (if required), WNV testing, data collection and reporting, and program assessment. These are all addressed in this guide. The surveillance elements of the plan allow preventive medicine personnel to assess local WNV risk. Regardless of how a WNV program is implemented, each installation should establish liaison with their local (civilian) mosquito abatement or public health agency to notify them of their WNV plan and to share information with them.

4. Assessing WNV Risk

Local risk is assessed through surveillance and testing. There are a number of established markers that are associated with risk of local transmission of WNV to humans, and these are all addressed below. A chart that translates surveillance and test data into risk categories is presented in Appendix B. This chart also recommends preventive or corrective actions (or inaction) at the various risk levels.

Not all installations will have the same degree of WNV risk. It may be that an installation can adequately perform WNV risk assessment by coordinating with a surveillance program conducted by a local (civilian) health department or mosquito abatement district. For instance, a given installation may be able to use arbovirus testing data or sentinel chicken flock results from nearby collections conducted by a county or State

health department or local university. Many states now have a dead bird surveillance program in place and often post results on their Internet website. If there is sufficient information that can be obtained from such liaisons, and if mosquito populations are considered low, then the installation may be adequately covered by WNV surveillance conducted by these civilian agencies. However, it is the responsibility of each installation to ensure that proper surveillance data is obtained, whether it is from the military medical department, public works department, pest management contractor or local or State public health agencies. This data should be reported periodically as described in the installation WNVSPP. Reporting procedures for all aspects of the plan are addressed in their respective sections below and summarized below in Section 8.

5. WNV Surveillance

The plan you produce and enact should include surveillance for the virus itself. WNV surveillance seeks to provide data that allows the assessment of local risk of human infection with WNV. Mosquitoes are captured alive and tested for the presence of the virus; and other animals, particularly birds, are monitored for exposure, illness or death due to WNV infection. Positive results through any of these methods is clear evidence of the presence of WNV locally, but negative results are no assurance that the virus is not present. The mere presence of the virus is not a measure of risk. Once the results of this surveillance begin to accrue, consult the risk matrix in Appendix B to see how they are to be interpreted into action.

a. Who Will Conduct WNV Surveillance?

Your plan should state which of the following options, alone or in combination, will be employed:

(1) Local Public Health and/or Mosquito Control Agencies. If local, county, or State agencies are currently performing surveillance, testing and control measures that meet or exceed U.S. Navy WNV recommendations, then arrangements may be made with that agency to include the military installation as part of the local agency's operations. Data should be reported to DVECC Jax for installations east of the Mississippi River and to DVECC Bangor for those west of the Mississippi.

(2) Private Contractor. The installation may develop and implement a contract with civilian mosquito controllers that meets or exceeds U.S. Navy WNV recommendations. This would be recommended in cases where mosquito surveillance is already done by contract, but the contract would need to be modified or re-written to include the elements unique to WNV surveillance. Data should be reported as in 5.a.(1)

(3) DoD Personnel. Installations may use DoD medical personnel and equipment to implement the WNVSPP. Data should be reported as in 5.a.(1)

b. Gravid Trap Collections. Gravid traps collect wild, blood-fed mosquitoes that are ready to lay eggs (that is, they are gravid.) It captures them alive, which is absolutely necessary for the subsequent testing they will undergo. No males or unfed mosquitoes are captured, and few non-mosquito insects are attracted to the trap because of the nature of the lure used. For WNV surveillance, the gravid trap should be the primary surveillance tool for adult mosquitoes, as this trap targets blood-fed, female *Culex* mosquitoes, which are more likely to harbor WNV than mosquitoes trapped randomly from the local population. Appendix C provides detailed instructions on the use of the gravid trap. The number of traps used will depend on the size of the installation, but a minimum trapping program for any installation should include at least five gravid traps on smaller installations and 10 on larger installations, with traps operated at least two nights per week. Larger installations, those with housing, those with a greater variety of habitats, or those located in areas of active WNV transmission may need to have a greater trapping effort. Trapping effort should be sufficient to trap mosquitoes from all areas of concern (e.g. housing, bodies of water, horse stables, public use areas, wet areas), especially targeting *Culex* species as the species of concern in surveillance programs. Adult surveillance should begin about when the first generation of adults appears in spring and continue until the first hard frost in the area. Gravid traps are also useful as a mosquito surveillance tool: if gravid trap collections remain below 25 *Culex* mosquitoes per trap per night, the risk of WNV circulation in that area is currently considered low. Assistance in establishing and interpreting a gravid-trap surveillance program can be obtained from Navy Entomologists at cognizant DVECCs and EPMUs. You are strongly encouraged to contact DVECC Jax or DVECC Bangor prior to establishing any WNV mosquito surveillance program on your installation.

c. Other Traps for Adult Mosquitoes. Mosquitoes collected in other traps, such as New Jersey Light Traps, may not be captured alive, and so cannot be used for WNV testing. Mosquitoes usually do remain alive and viable for WNV testing if collected in SSAM or CDC light traps. The large number of unfed mosquitoes, male mosquitoes, and "trash" insects captured in light traps and CO_2 traps makes the processing of the catch much more difficult, and reduces tremendously the percentage of WNV-positive individuals in the catch. While useful for *mosquito* surveillance, these other traps should not be substituted for WNV surveillance.

d. Processing Mosquitoes for WNV Testing. Each laboratory may have different requirements for the processing and delivery of female mosquitoes for WNV testing, and their requirements may change as improvements are made to the testing process. *Use the protocol that your laboratory sends*. It may differ in detail from the example given here, but the main ideas are still the same. Female mosquitoes collected for WNV analysis should be quickly immobilized by placing in a freezer and thereafter always kept at freezing temperatures. All identification work needs to be done on a chill table or an improvised equivalent that keeps the specimens in contact with a freezing surface. (See Appendix D for an example of an improvised chill plate.) The methods currently used to test female mosquitoes for WNV infection require that the mosquito specimens do not degrade due to warm temperature. Therefore a "cold chain" must be maintained at all times to keep the mosquitoes viable for testing – they must always stay frozen. Some new protocols place less emphasis on the cold chain, so again you are directed to follow the protocol of your testing laboratory. Once identified to the level required in your lab's protocol, the specimens should be placed into vials (preferably cryovials) based on the species (or the genus, depending on your laboratory's protocol.) Each vial is considered a "pool," therefore a pool of female mosquitoes is all specimens collected of one species, up to 25 individuals. (Some protocols allow up to 50 individuals in a pool.) For instance, at a given installation, collections from a night of trapping are as follows:

Aedes vexans	80
Culex tarsalis	5
Ochleratatus taeniorhynchus	39

Assuming that your protocol states that a pool may only contain up to 25 specimens of each species, the *Aedes vexans* will form four pools (three pools of 25 and one pool of five), the *Cx. tarsalis* will form one pool (five mosquitoes), and the *Oc. taeniorhynchus* will form 2 pools (one pool of 25 and one of 14). Your catch from that night will be submitted to the lab in seven pools. Female mosquitoes must be pooled from only a single night of trapping; DO NOT combine specimens from more than one night to increase the numbers of mosquitoes in your pools. Once identified and sorted into pools, mosquito should be kept frozen at -20ºC until shipped for analysis. The specimens must stay cold during shipping, as well, and your laboratory will guide you in this. Again, ALWAYS follow the protocol provided by your laboratory, even if it conflicts with information in this guide.

In 2003, testing of mosquitoes collected from Navy and Marine Corps installations in the Eastern US (east of the Mississippi River) is being provided by the US Army Centers for Health Promotion and Preventive Medicine (CHPPM) located at Ft. Detrick, MD (CHPPM-North) or Ft. McPherson, GA (CHPPM-South). Testing of mosquitoes collected from installations in the Western U.S. is being provided by CHPPM-West located at Ft. Lewis, WA. Contact information can be found in Appendix A. You **MUST** contact these agencies before shipping any specimens. They will provide the up-to-date guidance needed to assure that the specimens are handled, recorded and shipped properly. The Operations Department at either DVECC will assist you with this process if needed.

Human WNV cases should be reported directly to the Navy Environmental Health Center (NEHC) CDO at (757) 621-1967. Suspected human WNV cases must be diagnostically confirmed by testing serum or cerebrospinal fluid (or other appropriate tissue) and results reported to NEHC.

e. Dead Bird Surveillance. Bird morbidity and mortality has been found to be the most sensitive and cost-effective method for detection of WNV in an area. Your WNVSPP should state that any local dead bird, for which the cause of death is unknown, will be reported to installation veterinary staff who should be prepared to recover, assess, and prepare the bird properly for WNV testing by either a federal or state laboratory. In the absence of a military veterinary unit, contact your DVECC for guidance on this portion of your plan. The surveillance factor most closely associated with the number of human cases is *dead crow density* (number of dead crows per square mile), and most of the WNV positive birds reported have been dead American Crows. However, many bird species have been found to harbor WNV, and reporting dead birds should not be limited to crows. In fact, collection data from 1999-2001 show that WNV confirmation in an area could be delayed weeks if only dead crows were reported. Therefore, other types of birds found on the installation, those for which the cause of death is not known, should be reported.

f. Sentinel Flocks. Sentinel flocks are birds, usually chickens, that are caged outdoors for the purpose of detecting the presence of mosquito-borne encephalitis viruses in the area. The birds, which are known to have never been exposed to these viruses, are maintained so they can be bitten naturally by local mosquitoes. Their blood is periodically sampled and tested for antibodies to determine if mosquitoes have infected them with EEE, SLE or WNV. A certain number of these positive "seroconversions" is expected if any of these diseases is endemic to the area, and the experienced public health official can use the information to help judge the level of human risk.

The Navy does not maintain sentinel flocks for viral surveillance, but civilian health authorities in your area may do so. If so, you should use their data and expertise to help estimate the risk of human infection with WNV on your installation.

6. Mosquito Management

A thorough discussion of mosquito management is beyond the scope of this publication. In some areas of the country, mosquito management is a highly complex, integrated program requiring the attention of many technicians with specialized training and experience. This section is included in this document because a safe, effective and environmentally sound mosquito management plan is an integral part of any WNVSPP. Because there is no vaccine or cure for WNV, mosquito management may be the primary tool available to reduce the incidence of human disease.

Most programs require that all mosquito management operations be conducted by personnel certified in EPA category 8, "Public Health Pest Management." At a minimum, the certification is required for personnel applying pesticides, interpreting surveillance results, and advising on the conduct of pesticide applications. Military members should be certified through the Department of Defense program, while contract workers may hold the appropriate State certification.

Mosquito management programs, which are defined precisely for your installation in your local Installation Pest Management Plan, consist primarily of surveillance, non-chemical control measures, and chemical control measures. In areas where there is transmission of human disease, the IPMP's Emergency Vector Control Plan may include additional public relations requirements. Your WNVSPP should draw its mosquito management information from these two local documents, and it should contain (at least) the following elements.

a. Mosquito surveillance. A thorough program surveys for both immature and adult mosquitoes. Mosquito breeding site identification should begin during early to mid spring by surveying the installation and noting actual and potential breeding sites on a base map. Global Positioning System (GPS) and Global Information System (GIS) tools should be used whenever possible to document mosquito breeding areas on the installation accurately. Breeding sites may or may not have larvae at the time of survey. Periodic surveillance (typically weekly) should commence after the first generation is found in breeding sites. Larval surveillance should continue throughout the mosquito breeding season, because the species composition of the mosquito

populations often changes within a single breeding site over the course of the mosquito breeding season. Surveillance should include collection (done with a simple plastic or enamel dipper on the end of a 3-4 foot pole), identification of larva, mapping and monitoring of larval sites on the installation, looking for new sites as weather or other conditions change, and keeping thorough records of findings. While immature mosquitoes can be identified to species in some cases, many personnel prefer to identify the adults. Immature mosquitoes can be reared to adults in larval rearing chambers (NSN 3740-01-454-2345) and the adult mosquitoes identified upon emergence. Effective larval surveillance is best carried out by trained, experienced and interested personnel.

Surveillance for adult mosquitoes can be conducted using light traps such as the Solid State Army Miniature (SSAM) Hock Model 1012 (NSN 3740-01-106-0091), the CDC model 512, and baited CDC gravid traps, available through commercial entomological supply sources such as the John W. Hock Co. (http://home.acceleration.net/jwhock/) or Bioquip Inc. (http://www.bioquip.com/). An effective surveillance program for adult mosquitoes in WNV-positive States should use a combination of light traps and gravid traps. The SSAM or CDC light traps should be used with 4-5lbs of CO_2 (dry ice) per night and can be used without the light source to avoid capture of large number of insects other than mosquitoes. The lure for gravid traps is discussed in Appendix C.

Complaint calls are another element used in adult mosquito surveillance, as are the results of seeking adult mosquitoes in their daylight resting sites. Data from different surveillance methods are often combined to determine if mosquito activity exceeds some pre-determined action threshold for control efforts. Again, mosquito surveillance is best conducted by trained and experienced personnel.

b. Non-Chemical Control Measures. Minimizing the number of sites in which immature mosquitoes can develop is a fundamental control measure for reducing, or even eliminating, mosquito populations. Elimination, reduction or insecticide treatment of mosquito breeding sites is more efficient and cost effective than targeting adults, since immature mosquitoes are concentrated to a greater extent than in the adult stage. By finding and eliminating or treating potential larval sites, mosquito populations, and therefore the WNV threat, can be reduced or eliminated before virus transmission to humans occurs. Immature mosquitoes develop in any still water. Artificial containers that can fill with water, e.g., used tires or empty cans, should be discarded. Standing water should be drained and depressions that hold water should be filled when possible. Installation preventive medicine personnel may need to provide the local Public Works department a detailed work request to eliminate mosquito-breeding sites, especially those that may be eliminated by filling or draining. Larval mosquito surveillance should be scheduled for bodies of water that cannot be removed.

Larval mosquitoes can be controlled by mosquito-eating fish, which can be introduced into problem areas. These guppy-sized fish can be effective even in barrel-sized bodies of water, but they are not native throughout the U.S., so your environmental office should be consulted prior to their use.

For adult mosquitoes, effective use of screening is an important non-chemical step in reducing human exposure to bites. The public's use of repellents and the timing of outdoor activities are addressed below, under Public Relations.

c. Chemical Control of Mosquitoes. Several insecticides are available in the stock system to control immature mosquitoes, including "environmentally friendly" compounds such as *Bacillus thuringiensis* var. *israelensis* (Bti) briquettes (NSN 6840-01-377-7049) and insect growth regulators such as methoprene briquettes (NSN 6840-01-424-2495) or liquid (NSN 6840-01-424-2493). Because of the risk of environmental contamination when applying any type of pesticide to water, pesticide application should only be done by certified pesticide applicators in cooperation with installation Natural Resource Management and/or Environmental personnel. Entomologists at cognizant DVECCs or EPMUs can be contacted for assistance and consultation regarding mosquito control on Navy and Marine Corps installations.

Adult mosquito control is often done by ground ULV spraying, using pesticides such as sumithrin (NSN 6840-01-474-7751), pyrethrins (NSN 6840-01-104-0780) or resmethrin (NSN 6840-01-359-8533), and can be an acceptable and immediate way to reduce mosquito populations. Again, only personnel trained and certified in pesticide application should apply pesticides. Control of adult mosquitoes is generally based on trap surveillance data. An action threshold is set, and spray operations are conducted and continued until adult populations fall below the threshold. Often, insecticide applications conducted for 3 to 4 consecutive days/nights (and timed to coincide with daily peak activity periods of the target species) may be required to reduce mosquito populations to a noticeable degree. Entomologists at DVECC Jax or DVECC Bangor are available to provide technical assistance regarding adult mosquito control. For control of larger areas or when severe outbreaks occur, aerial spray operations may be considered. Entomologists at cognizant DVECCs must be contacted if aerial pesticide application is being considered, as this requires validation by an Entomologist. It is important to note that **no mosquito control plan should rely solely on spraying adulticides in response to mosquito complaints**. Surveillance, breeding site reduction and larval control measures should continue until the first killing frost occurs.

7. Public Relations. Informing the general public and maintaining active liaison with supporting communities (public health departments, health care providers, veterinary communities, etc.) are critical for the effective implementation of any WNV surveillance and control plan. News bulletins and interviews, literature, web sites, and even articles submitted to base newspapers should be considered to inform local military and other DoD personnel of the risks and precautions associated with WNV. Tab Q of the USACHPPM WNV plan contains examples of several public notices (http://chppm-www.apgea.army.mil/ento/westnile/South/Cover.pdf). The public should be made conscious of the risk when the risk level is high. They should be informed about limiting activity outdoors, especially from dusk to dawn, and they should be informed on the proper use of repellents for both the clothing and the skin.

8. Summary of Compliance and Reporting Requirements

a. Have a written WNVSPP, drawn from the IPMP and EVCP. The WNVSPP should also include:

    1) Protocol for gravid trap surveillance
    2) Protocol for submission of mosquitoes for WVN analysis
    3) Protocol for dead bird surveillance

b. Maintain a log of mosquito surveillance activity (see Appendix E for an example)
c. Maintain a dedicated log of mosquito pools sent for analysis (see Appendix F for an example)
d. Report results of mosquito pool analyses to cognizant DVECC and NEHC, if not tested at DoD laboratory (See Appendix G for an example)
e. Report results of dead bird analysis to cognizant DVECC and Installation Pest Management Coordinator (See Appendix H for an example.)
f. Report time and resources spent on program via current pest management reporting system

Appendix A.

Contact Information

Navy Environmental Health Center, Portsmouth, VA (NEHC):
757-953-0717, (DSN 377)

Navy Disease Vector Ecology and Control Center, Jacksonville, FL (DVECC Jax):
Officer in Charge (OIC), 904-542-2424 (DSN 942) cmd@dveccjax.med.navy.mil
Operations Department (904) 542-2424 DSN 942-2424.

Navy Disease Vector Ecology and Control Center, Bangor, WA (DVECC Bangor):
Officer in Charge (OIC), 360-315-4450 (DSN 322); mail@ndvecc.navy.mil
Operations Department (360) 315-4452, DSN 322-4452

Navy Environmental and Preventive Medicine Unit Number Two, Norfolk, VA (EPMU-2):
(757) 444-7671 or DSN 564-7671

Navy Environmental and Preventive Medicine Unit Number Five, San Diego, CA (NEPMU-5):
(619) 556-7070 or DSN 526-7070

US Army CHPPM-North:
ARMY CTR FOR HEALTH PROMOTION
& PREVENTIVE MEDICINE (ATTN: WNV Testing)
ENTOMOLOGICAL SCIENCES DIVISION
BUILDING 4411, LLEWELLYN AVE.
FORT GEORGE G MEADE MD 20755-5225
(301) 677-3932, ext. 3466/3806

US Army CHPPM-South:
ARMY CENTER FOR HEALTH PROMOTION &
PREVENTIVE MEDICINE (ATTN: WNV Testing)
ENTOMOLOGICAL SCIENCES DIVISION
Bldg 180, 1312 Cobb St SW
FT McPherson, GA 30330
(404) 464-3332

US Army CHPPM-West:
ARMY CENTER FOR HEALTH PROMOTION &
PREVENTIVE MEDICINE (ATTN: WNV Testing)
ENTOMOLOGICAL SCIENCES DIVISION
Bldg 9030, 5th and Blaine
FT Lewis, WA 98433-9500
(253) 966-0163

WNV Program Links:
CDC WNV Surveillance Program:
http://www.cdc.gov/ncidod/dvbid/westnile/resources/wnv-guidelines-apr-2001.pdf
USACHPPM WNV Mosquito Surveillance Program:
http://chppm-www.apgea.army.mil/ento/westnile/South/Cover.pdf

Appendix B – West Nile Virus Risk Levels and Recommended Actions

WNV Transmission Potential		Conditions	Action
none	**Level 1A**	Off season, mosquitoes inactive, climate unsuitable	Determine if new WNV Plan measures have been issued, confer with local agencies
	Level 1B	Early spring, adult mosquitoes not active	Begin larval breeding site mapping, surveillance, and source reduction
	Level 1C	Adult mosquitoes active but not problematic (no complaints),	Begin adult surveillance
low	**Level 2A**	Surveillance finds adult mosquitoes active, but no complaints	Public education and personal protection information should be provided
	Level 2B	Complaints of mosquito biting or mosquito traps collecting numbers of mosquitoes but no WNV found on installation	Consider adult/larval control measures, increase mosquito and dead bird surveillance, use ground truth maps for controlling breeding sites
	Level 2C	Dead bird(s) found where cause of death is unknown	Have installation vets or local public health agency test for WNV, increase surveillance for dead birds, public announcement about what public should do if a dead bird is found
med	**Level 3**	WNV confirmed in dead bird(s) locally or 1 or more WNV+ mosquitoes in traps	Consult with local mosquito abatement agencies & DVECCs, get public involved with environmental sanitation, consider increased adulticides and/or change in adulticide(s)
high	**Level 4**	1 or more WNV+ person or 1 or more WNV+ horse in area	Greater public announcement, consider wider use of adulticides esp. in public use areas, consult with local mosquito abatement agencies, consult with DVECCs

Note: Once an activity is started, it should be continued until the end of season or risk decreases.

Appendix C - Guidance for Use of the CDC Gravid Trap

U.S. Army Center for Health Promotion and Preventive Medicine – North, 30 May 2001

1. **Purpose.** To determine the relative human health threat due to West Nile Virus (WNV) by detecting the presence of arboviral agents in female mosquitoes, primarily *Culex* spp., that have taken a blood meal and are searching for an oviposition site.

2. **General**. The occurrence of human and animal illness due to WNV in New York in the fall of 1999 demonstrated the necessity for the early detection of this and other arboviruses of human health concern. Although adult mosquito light trapping using standard issue light traps (e.g., Solid State Army Miniature [SSAM] traps [a.k.a. CDC traps] or the new Standard Miniature Light Trap [SMLT]) can detect vector species and, with the application of appropriate procedures, can collect specimens that can be tested for viral pathogens, these traps also collect unfed (i.e., uninfected) mosquitoes as well as male (non-biting) mosquitoes and non-mosquito arthropods. The CDC gravid trap is selective for female mosquitoes and is, therefore, a more sensitive method for evaluating the arboviral threat in a given area.

3. **Equipment**. The CDC gravid trap is available through the National Stock System. The current information is as follows: Item – CDC Gravid Trap, NSN Part Number – 1712, Unit Price - $88.00. It is also available through commercial entomological supply sources (web search using key words "mosquito trapping supplies" or "entomological surveillance supplies.") See figure 1 for trap components. Batteries and chargers must also be ordered.

4. **Timing of Gravid Trapping Program**. In the northeast, trapping should commence not later than 15 June. Historically, arboviral-positive mosquitoes have been detected in this region by the first of July. If positive birds are detected earlier in a given area, start earlier. Ideally, gravid traps should augment an already-existing light trapping program that commenced in May or early June. Light trapping can be valuable in determining where gravid traps should be placed.

5. **Trap Site Selection**. Locate the trap in or near residential areas to collect container-breeding *Culex* spp. Traps should be located in areas protected from extreme environmental conditions (e.g., wind and direct sun) and in secure areas (not conspicuous) where they are not disturbed or vandalized. Appropriate trap sites include utility yards, window wells, stairwells, storm drains, boat yards, animal stables, transformer pits, cluttered backyards, tire storage yards, sewage treatment plants, near garden plots, and in cemeteries. It is desirable to have some type of overhead cover (e.g., shrubs or overhangs) so the tub is not easily flooded in the event of rain. Locate traps where they can be visited daily. If, after several visits, the trap does not appear productive, move it to another location. Remember the primary goal is to collect blood-fed female mosquitoes. It is less of a priority to maintain consistent sites having poor yields. Ideally, six gravid traps should be put out on an average-sized installation. Traps should be spaced at least several hundred yards apart.

6. **Trap Setup**. If using the trap for the first time, season the plastic tubs to rid them of insect repellent properties associated with chemicals in some plastics. Immerse them in a muddy pond for a few days. At least two days before trapping, make a concentrate by mixing about one cup of rabbit pellet food per one gallon aged water. Let the mixture incubate in a location protected from mosquitoes. At the trapping site, put about ¼ gallon of the concentrate into the tub and add aged water collected from a nearby natural source or brought along with you to bring the water level in the tub up to about 2 inches from the bottom edge of the fan housing tube. Position the trap bracket securely over the center of the tub and slide the collection bag over the top of the trap tube. Be sure the bag is not askew and that it remains properly positioned, even if breezes pick up. Attach the battery to the terminal wires ands make sure it is securely positioned, and test the trap making sure the fan turns freely and draws the air from below. If the fan is spinning in the wrong direction, reverse the wires on the

battery terminals. If the trap is not equipped with an automatic timer, place it out in the late afternoon so the battery will run through the evening. Assign the trap a number and note its location on a map.

7. **Trap Servicing and Specimen Collection**. Visit the trap in the early morning the following day. Carefully remove the trap bag containing mosquitoes and replace it with an empty one. Tie off the open end, and if the bag is not easily hung in the servicing vehicle, place net props (e.g., tongue depressors) around the bag so it does not collapse, crushing the mosquitoes. Record the general number of mosquitoes taken from each trap (to be verified in the shop later) and any other relevant information. The water can be used for multiple trap nights within a week. Top it off with aged water as needed to make up for evaporation. After one week in the pan, dump the water and replace it as described above. Don't allow the old water to stand for more than a week, as it will breed mosquitoes. Before dumping the water, you may want to collect any visible eggs and rear them to confirm species identifications.

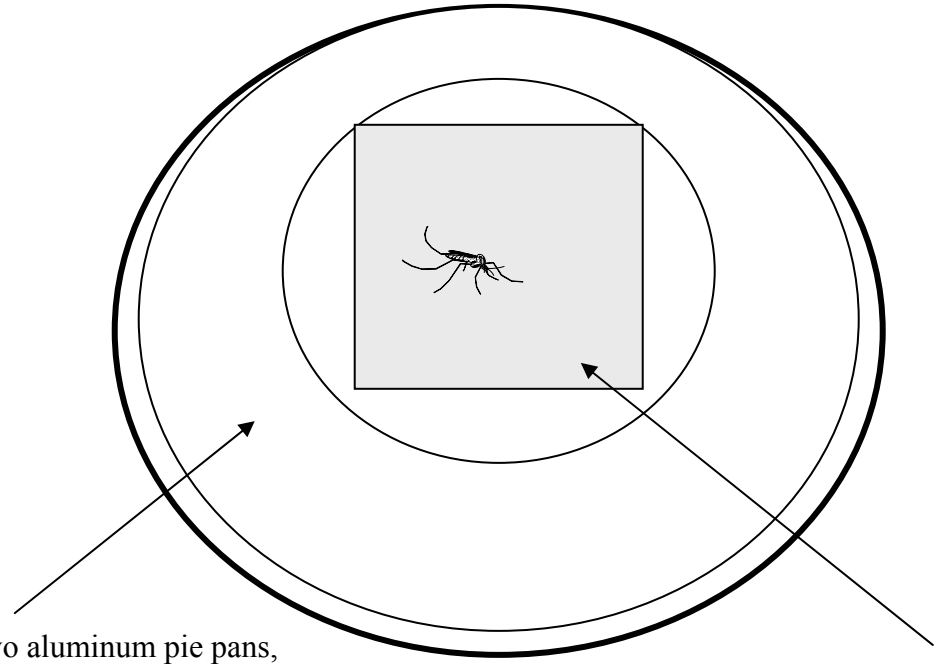
8. **Specimen Handling**. Do not expose captured mosquitoes to direct sunlight or extreme temperatures as may be generated in a closed vehicle. The mosquitoes must be alive for viral testing. Captured mosquitoes may be transferred from the net bag to a more compact handling container (e.g., paper ice cream container with a rubberized aperture) using a portable, powered aspirator or a HEPA-protected oral aspirator (Figure 2.) Upon returning to the laboratory, place the handling container or the entire bag containing mosquitoes in a normal freezer (<32º F) for at least 30 minutes or in an ultra low freezer (<-60º F) for at least 15 minutes. Empty the frozen mosquitoes onto a chilled surface such as a chill table or an enamel pan nested in an ice bath. Examine the specimens with a hand lens or a dissecting microscope. Sort, package and ship the specimens in accordance with the viral assay protocol you are using. Record all required data and make sure the pool vials are clearly labeled.

9. **Weekly Schedule**. Trap at the beginning of the week, Monday, Tuesday and Wednesday, to allow time for sorting and express mailing specimens before the weekend. This also allows time for adjustments if for some reason you can't trap on a given night. Remember, the attractant concentrate needs to incubate for at least two days, so it should be prepared on the previous Friday for the following week.

Fig. 1. Gravid Trap

Fig 2. Transferring mosquitoes using a HEPA-filtered aspirator

Appendix D – Improvised cold plate used to keep specimens frozen while sorting and identifying.



Two aluminum pie pans,
stacked, with water frozen between them

Filter paper to absorb condensation

A chill table can also be used to sort mosquitoes. Chill tables can be purchased through Bioquip, Inc (http://www.bioquip.com/newproducts/1429.htm).

Appendix E.

Example of logbook entry for mosquito surveillance documentation

Date	**Collection Time**	**Method**	**Location**	**L**arval/**P**upal/**A**dult	#♀ / ***Genus***	**Collector**
25-26 Apr '02	1800-0600	CDC Lt & CO_2	behind hospital	A	17 *Culex*	HM3 Smith
25-26 Apr '02	1800-0600	CDC Lt & CO_2	behind hospital	A	4 *Aedes*	HM3 Smith
25-26 Apr '02	1800-0600	CDC gravid	walking trail pond	A	23 *Culex*	HM3 Smith
25-26 Apr '02	1800-0600	CDC gravid	horse stable pond	A	16 *Anoph*	HM3 Smith
28-29 Apr '02	1800-0600	CDC Lt & CO_2	behind hospital	A	20 *Culex*	HM2 Darwin
28-29 Apr '02	1800-0600	CDC Lt & CO_2	behind hospital	A	8 *Aedes*	HM2 Darwin
28-29 Apr '02	1800-0600	CDC gravid	walking trail pond	A	23 *Culex*	HM2 Darwin
28-29 Apr '02	1800-0600	CDC gravid	horse stable pond	A	17 *Anoph*	HM3 Smith

Appendix F.

MOSQUITO POOL INFORMATION **Installation:** _____

WEST NILE VIRUS SURVEILLANCE **State:** ____ **County:** _____

Submitter: _____

Phone & e-mail: _____

Pool Log No.	Collect. Date	Trap Site	Collect. Method	No. of Mosq.	Mosquito ID	Notes/ Comments	Lab *No.*	*Test Date*	*Test Result*

Lab Use Only

Pool Log No.:	**Use an installation code prefix & numeric suffix (e.g., FGGM001, FGGM002, etc. for Fort Meade)** Important - make sure the vial is clearly labeled with the same log # (written on cap and on side).
Collection Date:	mm/dd/2000. For consistency, use the date the traps were PUT OUT
Trap Site:	Use your own number/code scheme. Make sure you know how to link it to a site on map
Collection Method:	Light Trap w/CO2 (LTC), Light Trap w/out CO2 (LTX), Gravid Trap (GT), Resting Collection (RC), Other (O) and specify in notes.
No. of Mosquitoes:	Total number within the pool. If zero, enter "0" but DO NOT ASSIGN A POOL # (use a "-")
Mosquito ID:	*Culex* (Cx), *Aedes* (Ae), *Anopheles* (An), Other (O), if you know the species put it down
Notes:	Anything worth mentioning
Shaded areas:	Leave blank
Further Tips:	It is OK to use solid continuation lines for information that is redundant within a column. It is OK to draw a squiggly line through a row to divide different trap nights

Only pool FEMALE mosquitoes. DO NOT USE DUPLICATE POOL NUMBERS. **Appendix C. West Nile Virus**

Appendix G.

Weekly Mosquito Testing Report

COLLECTION WEEK:

Date	Installation / State	# Trap Nights	# Collected				# Pools Sent	Results
			Aedes	*Anopheles*	*Culex*	Other		

An example data sheet for reporting WNV mosquito testing results to DVECC Jax or DVECC Bangor if WNV surveillance and testing is not done by DoD personnel.

Appendix H.

Vertebrate WNV Testing Data Sheet

COLLECTION
WEEK:

		Humans		Avian				Mammals				
								Horses		Other		Notes
Date	Installation	Tested	Result	Bird species	#sick/dead birds collected	# Tested	Result +/-	# Tested	Result	# Tested	Result	

An example report form for reporting vertebrate WNV testing results to the installation Pest Management Coordinator, and DVECC Jax and DVECC Bangor.